Journal of KerbalaUniversity , Vol. 11 No.1 Scientific . 2013

Modification of ultrasound cavitation effect induce changes in the cell redox functional activity by means of chemiluminescence.

التأثير الفجوي للموجات فوق السمعية على الفعالية الحيوية التأكسديه للخلايا مقاسه بطريقة اللمعان الكيمياوي

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Abstract

The cavitation effect of ultrasound (U S*) in vivo* situation can be monitored *in vitro* by the changes in the redox activity of yeast cells using ***lucigenin dependent chemiluminescence*** (CL). This technique can be used to detect the cavitational harmful effects of different ultrasound frequencies used in medical diagnosis.CL of yeast cells is produced by excitation of lucigenin on the oxidative free radicals and hydrogen peroxide, in the suspension medium. The changes in the redox activity was monitored at different viscosities of the medium. The results suggest that increase in the viscosity of the suspension medium reduced the cavitational events. This appear clearly as the(% CL) decreases significantly($P<0.05$) with decreasing the viscosity of the suspended medium equivalent to greater than(0.3gm/100ml).

Keyword: Ultrasound, chemiluminescence, yeast, redox activity.

الخلاصة : -

إن التأثير البنيوي للموجات فوق السمعية على الخلايا الحية يمكن مراقبته مختبريا على تغيرات فاعليه الاكسده والاختزال لخلايا الخميرة باستعمال اللمعان الكيماوي المنوط بالوسجنيين . أن اللمعان الكيميائي لخلايا الخميرة ناتج عن استثاره اللوسجنيين بالمستقلب المؤكسد الماء الاوكسجيني في الوسط المعلق. وتم مراقبه التغيرات في فاعليه الاكسده والاختزال لأوساط مختلف اللزوجة . وتقترح النتائج بأن الزيادة في لزوجه الوسط المعلق يقلل من التأثير الفجوي للموجات فوق السمعية . حيث ظهرت أن النسبة المؤيه للمعان الكيماوي قد تناقصت بمقدار واضح ($P< 0.05$) مع نقصان كثافة الوسط المعلق للخلايا بكثافة أكثر من (0.3 gm / 100 ml)

Introduction

Several observations of non-lytic changes in mammalian cell membrane induced by US have been reported and described as the non-thermal effects of US , such as the shearing forces associated with stable cavitation which occur for cells suspended in normal non viscous medium. [1,2] Membrane permeability changes in cells insonated in suspension have been observed, and attributed to shearing effect of bubble streaming and oscillation of the plasma membrane.[3]This yeast cell model mirror approximately the in vivo situation. Cells in tissue are held rigidly, where in suspension are free to move, streaming out of high intensity regions, and able to change shape to accommodate stresses.[4] In in vitro situation, there are many cell-cell and cell-container collision and viscous forces exist due to different streaming velocities of the media and cells.[5]However, it is difficult to extrapolate in vitro results to the in vivo situation in term of damage to the biological tissue.

The work described in this paper is designed to answer the question as to whether US continuous wave interaction at lower intensity($3.2watt/cm^2$) induced changes in redox activity in yeast cells that can be modified by increasing the viscosity of the suspension medium.

It has been demonstrated that increase in velocity of the US propagated medium can increase the intensity threshold at which cavitation is detected.[6] Although the existence of ultra weak luminescence in living biological system has been known for a long time, it becomes of interest in connection with the present work to develop a new approach for studying the effect of US on ultra weak luminescence of yeast cells (redox activity). This technique, using organic lucigenin as a probe for ultra weak luminescence signal amplifier is simple and sensitive method. So the aim of

the present study is to improve that ultrasound diagnosis image can be used in safe mode in both soft tissue and bone only, while the blood flow(Doppler) is recommend to use a constant media in fluid by using frequencies to improve results.

Methodology

Chemiluminescence of free radical is a method for detecting the record activity in this study, this was used to detect the reduction of cell activity due to the harmful effect of cavitation induced by US Shows block diagram of the apparatus we used to measure the light output from the biological system of yeast cells plus lucigenin. The optical system consisted of the cuvette mounted on light guide made of prespex rode of one inch diameter. This light guide element was located almost at entrance to the photomultiplier (PM) tube. The light emitted by the yeast cells plus lucigenin was received by a (50mm. diameter), (13) stage EMI 9635Q PM tube. The window passed light on to the bialkali(K-Cs) photocathod (figure. 1).

The (PM) tube was mounted inside a box and attached by means of coupling to the light tight chamber. The high potential required across the tube was provided by a stabilized (EHT) supply. The output from the anode of the (PM) tube was monitored by picoammeter. The picoammeter was used to monitor the DC-current output from the (PM) tube which was fed to an electronic chart recorder. The signal after current to voltage conversion was fed to frequency converter. The voltage frequency converter together with a multidecade counter act as a digital integrator.

Most of the measurements of the light intensity of the yeast plus lucigenin reactions were recorded in arbitrary units, in reference to the standard light source. The overall gain of the system remained constant. All experiments were carried out in cuvette of equal dimensions.

The suspension medium

The suspension medium consists of a simple ionic medium (simple salt solution) as follow: NaCl (7.20gm/ml), $NaHCO_3$ (1.925gm/ml), pH was maintained at (7.4), KCL (6.00gm/ml) and D-glucose (7.4gm/ml).

Yeast cells

Suspension of brewer's Batton yeast was used as a biological module, (0.5gm) of yeast was suspended in (50ml) of the suspension medium at 37C$^{\circ}$ in water bath for (15) minutes, (2ml) of the yeast suspended cells were taken and injected into cuvette containing (100µl) of lucigenin (0.1mg/ml). In each experiment conducted in this study, the amount of yeast was kept constant (5×10 cell/ml), pH(7.4), and a temperature at (37C$^{\circ}$). Agar at different concentrations was added to the simple salt solution to increase the viscosity of the suspension medium (table 1) ; the control (medium without agar) while the test (medium with different agar concentrations). Lucigenin, structure, and mechanism of action is shown in (figure.2).

Radiation pressure of ultrasound

Continuous wave insonation was carried out in thoroughly degassed distilled water in a water bath lined with absorbing rubber to prevent standing wave formation. The distance between the cells container and the horizontal transducer face was (4cm). Insonation was carried out using a US therapy equipment capable of providing an intensity of (3.2watt/cm^2 special average) at (0.8MHZ). The yeast cells were insonated at different viscosity of the medium in an open ended annular prespex container covered at both sides with melinex film(0.25mm)thick, window diameter (3cm). Cells were injected gently into the insonation vessel through a hole in the rim which was later sealed with a plastic screw. The cells in the prespex vessel were continuously agitated by a shaking mechanism to keep them within the beam and prevent settling for (60min). After insonation cells redox viability was carried out by adding (100µ) of lucigenin (0.1mg/ml) to (2ml) of yeast suspension and light intensity waveform of the yeast cells plus lucigenin was recorded at different viscosities of the media (figure.3).

Data analysis

Data were expressed as (mean ± SD), differences between control and tests groups were analyzed using (SPSS 9 version) using paired t-test, significant effect considered when the (p value < 0.05).

Results

The values reported in (table 1) indicate that the degree of lysis was reduced under conditions of increased viscosity at (3.2watt/cm^2). Cells insonated for different durations at (3.2watt/cm^2) in suspension of viscosity close to that of water shows change in the cell light yield (figure.3). The results exhibit a marked depression of amplified ultraweak luminescnece(redox activity) at low medium viscosity under same insonation condition.

Table(1) Influence of increasing viscosity of medium on redox activity light yield of yeast cells + lucigenin, at 0.8MHZ, 3.2W/cm^2 intensity, pH 7.2 and temperature 37c.

%CL Light yield ($\frac{\text{Test}}{\text{Control}}$ × 100)	No.5	Agar medium concentration (gm/100 ml)	
SD			P Value
Control ©(0.97) ± 0.131	5	0.5	1.000
T^1 (0.81) ± 0.153	5	0.4	0.113
T^2 (0.77) ± 0.191	5	0.3	0.089
T^3 (0.67) ± 0.172	5	0.2	0.014*
T^4 (0.56) ± 0.159	5	0.1	0.002*

Test(t): CL of sonicated sample at specific viscosity.
Control(c): CL of sonicated sample at water viscosity.
The CL results in the table were corrected for the same number of cells, and five readings at least.
*(CL%) decrease significally (P<0.05) with decreasing viscosity.

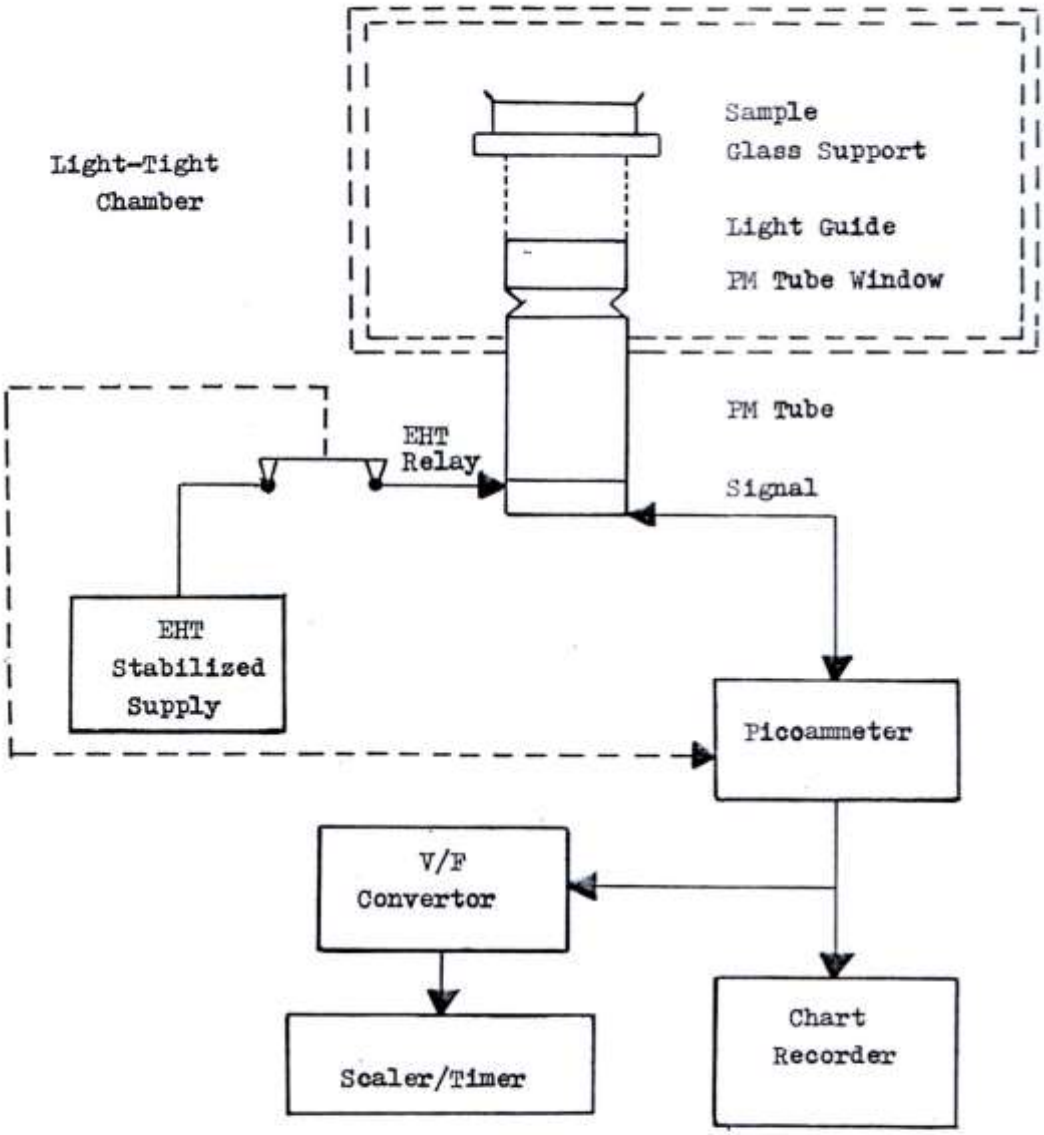

Figure. 1 Experimental apparatus.

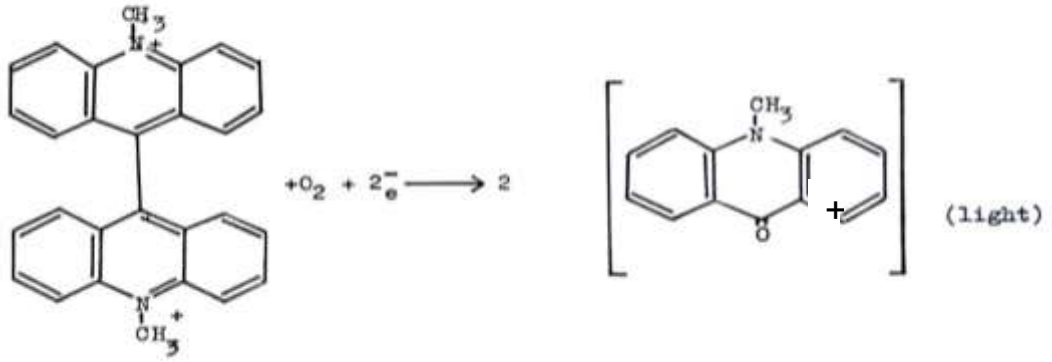

Figure 2.Generalized Lucigenin reaction: reductive,dioxygenation.(W.Adams and G.Gllento. 1982).

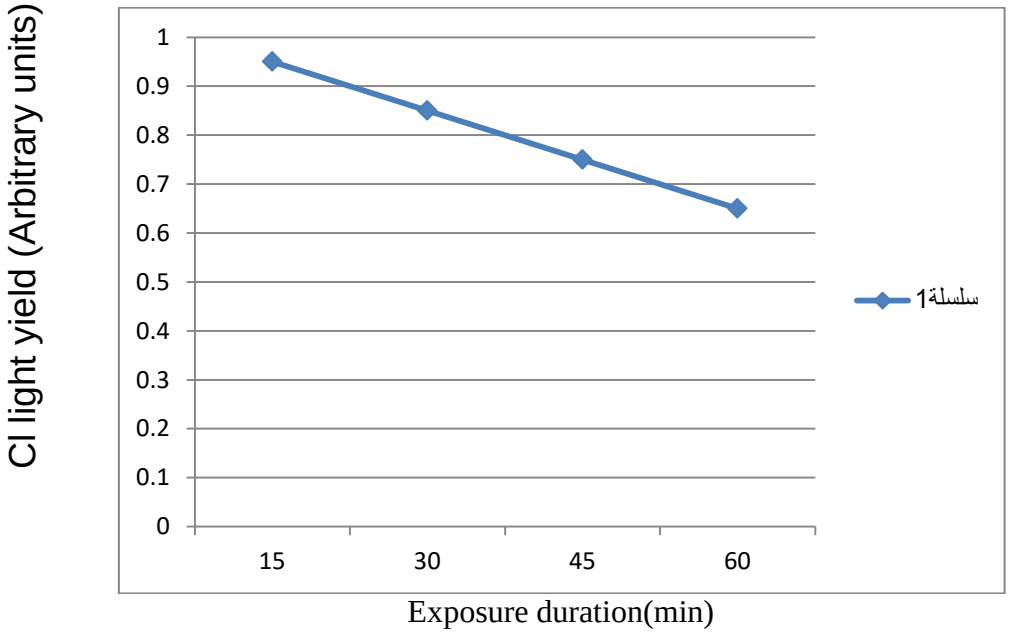

Figure 3. The relationship between exposure duration and CL light yield of cells+ lucigenin {area under CL waveform curve (Figure 4)}. Conditions : normal viscosity , pH = 7.4 , temperature 37c, ultrasound frequency 0.8 MHZ , and 3.2 Watt/ cm^2 intensity. Results are represented as mean± SD of three replicates.

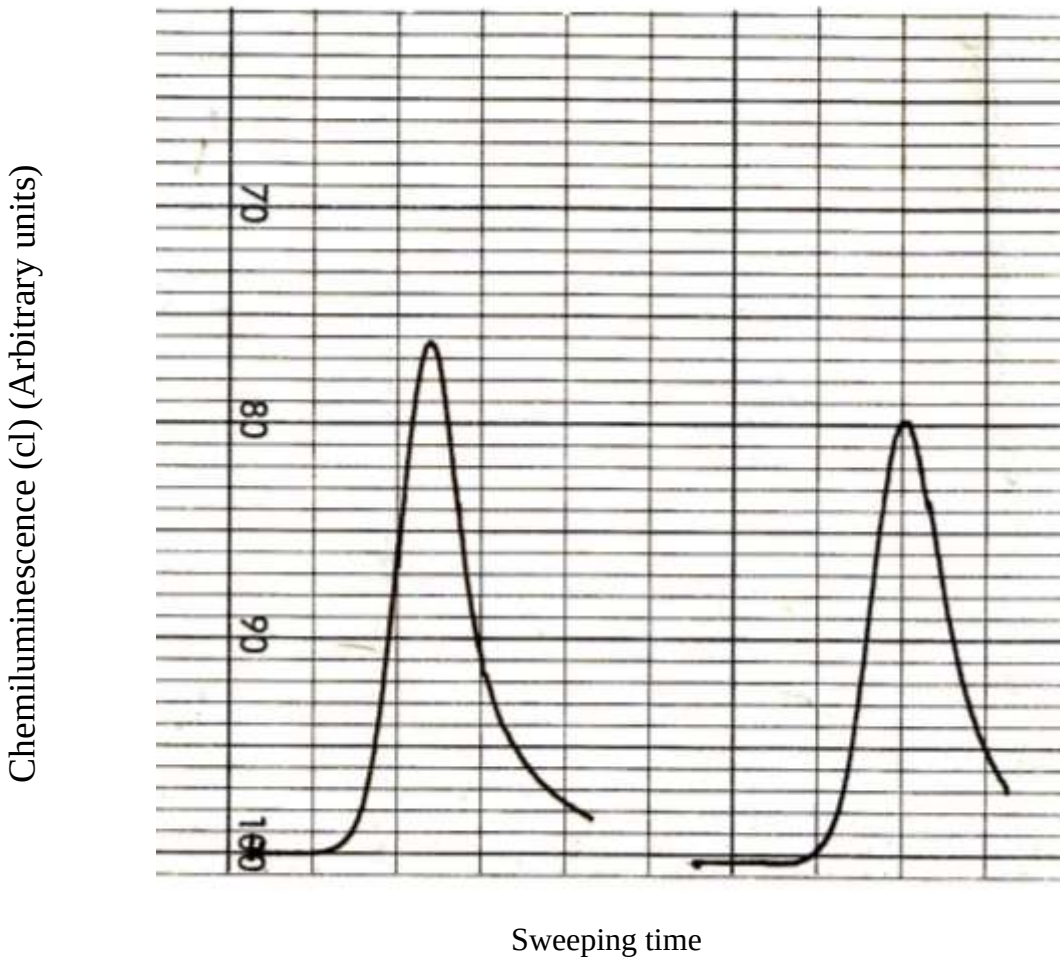

Figure 4. A typical CL Kinetic curve wave form .

Discussion

At the frequency (1-10MHZ) and intensity (0.3-0.5watt/cm^2) of current US therapy equipment, cavitational events have been shown to occur in water,[7] and in human blood plasma in vivo system .[8] In addition, the threshold for mammalian cells lysis in vitro has been reported to be between (0.5 and 1 watt/cm^2) at (1MHZ).[9]

In the present study, some cell lyses in the insonated suspension was detected and the degree of cell lyses increased at normal insonation condition of the suspended media (near the viscosity of water). On the other hand, the degree of lyses was reduced by insonating in media of increased viscosity. These findings suggest cell loss due to stable cavitational events, a conclusion supported by the detection of sonochemical effects, that induced change in redox activity of cells measured by means of CL. At normal viscosity the production of free radicals in a sonicated aqueous suspension appears to be contingent upon cavitational events.[10] It is thought that in an insonated aqueous suspension, free radicals are most likely produced outside the cell and must diffuse into the cell to interact.[11](figure3).

The US induced cavitation event, this cavitation occur at different US frequencies and directly proportional to the frequency.[12]The cavitation event harmful event may lead to cell membrane damage and rupture of the cells.[13]So as US diagnosis increasing the frequency of US for clear image(higher resolution), may lead to harmful event due to cavitation event increase probability.[14]This will limit the resolution in imaging to reduced the effect of cavitation especially in liquid (blood—blood flow Doppler measurement in US diagnosis).[15-16]The increasing in viscosity lead to reduced the effect of cell damage, due to reduced in cavitation event(soft tissue and bone) has high viscosity, the cavitation event reduced to minimum or has no cavitation.[17] So in

conclusion, the US diagnosis image of higher frequencies in soft tissue or bone is safe and recommended, while in blood is limited with safe frequency. The resolution in blood flow (Doppler) need contrast medium to compromise the reduction in low frequency(safe frequency).

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